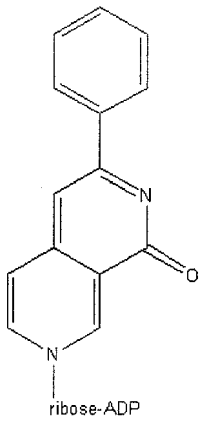
REMARKS

The amendment to claim 18 is supported by the application at the paragraph bridging pages 6 and 7. No new matter has been added. Entry of the amendment is respectfully requested.

Request for Reconsideration

Applicants would like to thank Examiner Bland for the courteous and helpful discussion held with Applicants' representatives on July 9, 2009. During the discussion, the fluorescent products disclosed in the references were discussed. Amending the claims to recite measuring the fluorescence of the fluorescent compound at 444 nm was suggested.

The present invention makes use of the discovery that treating a reaction mixture containing NAD+ with acetophenone in base, followed by heating during incubation with formic acid, yields compound **1**:



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Compound **1** is characterized by a strong fluorescence emission at 444 nm (page 6, lines 25-30). This allows for a fluorescent assay of NAD+ solutions having a concentration as low as 10 pM, and the fluorescence is linear over the range of 1 nM – 100 μM (p. 7, lines 5-8). Importantly, nicotinamide does not react under the assay conditions, and NAD+ and nicotinamide have no significant intrinsic fluorescence emission at 444 nm (p. 7, lines 8-11).

Rejections – 35 U.S.C. § 103

The rejection of the claims under 35 U.S.C. § 103(a) over Clark et al. (Analytical Biochemistry 68, 54-61 (1975)) in view of Osawa et al. (Journal of Clinical Microbiology, April 1997, p. 951-953), as evidenced by Nakamura et al. (Analytical Chemistry, Vol. 50, No. 14, December 1978, p. 2047-2051), and further in view of Pieper et al. (PNAS, February 15, 2000, vol. 97, No. 4, p. 1845-1850) or Hilton (Cancer Research 44, 5156-5160, November 1984) is respectfully traversed. The applied references, either alone or in combination, do not suggest the formation of a compound having significant intrinsic fluorescence emission at 444 nm.

When considering the differences between the prior art and the claimed invention, the prior art must be considered in its entirety, including disclosures that teach away from the claims. "A prior art reference must be considered in its entirety, *i.e.* as a whole, including portions that would lead away from the claimed invention." MPEP § 2141.02 (VI), citing *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

Clark et al. discloses a fluorimetric assay for N^1-methylnicotinamide (abstract). N^1-methylnicotinamide is converted to a fluorescent derivative by addition of acetophenone and KOH in 80% ethanol (page 61, second paragraph), followed by addition of 99% formic acid (page 56, second full paragraph). No heating following the addition of the formic acid is disclosed.

Excitation spectra were obtained with emission at 430 nm (Figure 3, legend). The nanomolar relative fluorescence of N^1-methylnicotinamide was observed to be about 4, while for NAD+ it was approximately 0.15 (page 56, last paragraph to page 57, first paragraph). The authors concluded that "NAD+ [...] yield[s] derivatives with a molar fluorescence about 1/25 that of methylnicotinamide" (page 61, second paragraph). No excitation spectra with emission at 444 nm by NAD+ derivatives were reported.

Nakamura et al. discloses a spectrofluorometric method for the determination of α-methylene carbonyl compounds by using N^1-methylnicotinamide chloride (NMN) (Nakamura et al., abstract). An alkaline mixture is formed by adding NaOH and NMN to an aqueous sample. After a period of time, the solution is acidified with formic acid (*Id.*, page 2047, second column, fifth paragraph). Nakamura et al. never carries out a

reaction with NAD+ as a reagent, and no excitation spectra with emission at 444 nm by NAD+ derivatives is reported.

Nakamura et al. discloses the production of a fluorescent product from N^1-mehtylnicotinamide. The formation of fluorophore is slow at 0 °C, faster at 50 °C, and yet faster at 92 °C, but no formation of side-products due to side-reactions occurring at 50 °C or 92 °C is reported (*Id.*, page 2049, second column, first full paragraph). The product of the reaction of Nakamura et al. is thus the *same* at 0, 50, and 92 °C, *i.e.* the temperature increase leads to a faster formation of the fluorophore, without giving rise to the formation of other products.

Osawa et al. discloses a method for identifying cholera-enterotoxin (CT)-producing *Vibrio cholerae* serogroups (abstract). The method includes measuring the concentration of NAD+ by means of a color-amplifying solution (paragraph bridging pages 951 and 952). The authors are entirely silent as regards fluorescent derivatives of NAD+. Pieper et al. discloses monitoring PARP activity through conversion of radioactively labeled [^{32}P]NAD+ to labeled PAR (page 1845, second column, last paragraph; page 1846, second column, second paragraph). No fluorescence-based methods are used or suggested. Hilton discloses a method for determining aldehyde dehydrogenase activity. The method includes measuring NADH fluorescent emission at 460 nm (Hilton, page 5157, second paragraph). Hilton, however, is silent as regards fluorescent derivatives of NAD+ having significant intrinsic fluorescence emission at 444 nm.

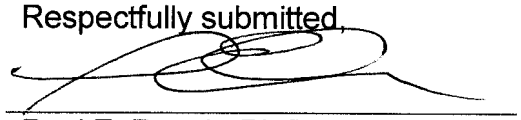
The applied references are silent regarding the formation of compounds having fluorescence at 444 nm. One of ordinary skill in the art would therefore not expect the formation of a compound that is strongly fluorescent at 444 nm; there would be no reason to measure fluorescence at 444 nm.

As claimed, the method of the invention comprises converting NAD+ to a fluorescent compound, and measuring an amount of fluorescence of the fluorescent compound at 444 nm. In view of the foregoing, the disclosures of the cited references would not have led one of ordinary skill in the art to carry out the claimed method. Accordingly, the claimed invention is not obvious over the applied references, and withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

Applicants respectfully submit that the application is now in condition for allowance. Early notice of such action is earnestly solicited. Should the Examiner feel a discussion would expedite the prosecution of this application, the Examiner is kindly invited to contact the undersigned at (312) 876-1400.

Respectfully submitted,



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